Yale - Total Cholesterol

John Stack¹, Gary Cline¹

¹Yale University

Mouse Metabolic Phenotyping Centers
Tech. support email: info@mmpc.org

Lili Liang

ABSTRACT

Summary:

Procedure used to determine the total concentration of cholesterol present in blood, serum, and plasma. Cholesterol esters are hydrolysed by cholesterol esterase. Cholesterol is then oxidized by cholesterol oxidase with formation of hydrogen peroxide. Peroxidase uses the hydrogen peroxide, phenol, and 4-aminotipyrine to form a quinoneimine dye which is measured at 500 nm.

DOI:
dx.doi.org/10.17504/protocols.io.y3rfym6

External link:

Protocol Citation: John Stack, Gary Cline 2019. Yale - Total Cholesterol.
protocols.io https://dx.doi.org/10.17504/protocols.io.y3rfym6

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Protocol status: Working
We use this protocol and it’s working
**PROTOCOL integer ID:** 21329

**Keywords:** Cholesterol

---

**MATERIALS**

<table>
<thead>
<tr>
<th>Material</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol Rapid Liquid Reagent</td>
<td>R85464</td>
</tr>
<tr>
<td>Multi Analyte Calibrator</td>
<td>R60010</td>
</tr>
<tr>
<td>Assayed Control Serum 1</td>
<td>R83082</td>
</tr>
<tr>
<td>Assayed Control Serum 2</td>
<td>R83083</td>
</tr>
</tbody>
</table>

**Reagent Preparation:**

- **Cholesterol Rapid Liquid Reagent:** As supplied by vendor
- **Multi Analyte Calibrator:** Add the appropriate amount of water (6.5mL) to the chemical control bottle. Invert to mix, allowing 15 minutes for the reagent to settle.
- **Assayed Control Serum 1:** Add the appropriate amount of water (6.5mL) to the chemical control bottle. Invert to mix, allowing 15 minutes for the reagent to settle.
- **Assayed Control Serum 2:** Add the appropriate amount of water (6.5mL) to the chemical control bottle. Invert to mix, allowing 15 minutes for the reagent to settle.

---

**BEFORE START INSTRUCTIONS**

**Analysis by automated system Cobas Mira Plus**

1. Calibrate Cobas for Total Cholesterol analysis by running a multi analyte standard and two control serum.

2. Sample handling as performed by Cobas Mira Plus.
   a) Pipette 3 µL of sample into a cuvette slot.
   b) Add 275 µL of Cholesterol Rapid Liquid Reagent.
   c) Mixture is incubated at 37ºC for 10 minutes.
   d) Absorbance is measured at 500nm.